TOXIC CYANOBACTERIA BLOOMS

A Field/Laboratory Guide

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INFORMATION ABOUT CYANOBACTERIA

WHAT ARE CYANOBACTERIA?

Cyanobacteria, formerly called "blue-green algae", are relatively simple, primitive life forms closely related to bacteria (both are prokaryotes) that should not be mistaken for true algae. Although they are typically much larger than bacteria, microscopic examination of cells reveals little internal structure. Nearly all are photosynthetic.

In addition to the lipid soluble chlorophylls and carotenoids, cyanobacteria contain a characteristic water-soluble pigment called "phycocyanin" which gives the group their blue-green coloration. When cyanobacteria blooms begin to die and disintegrate, this water-soluble pigment may color the water a distinctive bluish color. Depending upon the species, cyanobacteria can occur as single cells, filaments of cells or various colonial associations.

WHAT ARE CYANOBACTERIA BLOOMS?

Cyanobacteria are found throughout the world in terrestrial, freshwater and marine habitats. However, it is the freshwater habitat that typically experiences a cyanobacteria "bloom". Nutrient-rich bodies of water such as eutrophic lakes, agricultural ponds, or catch basins, may support a rapid growth of cyanobacteria. When conditions are good, a "clear" body of water can become very turbid with a green, blue-green or reddish-brown growth within just a few days. Many species can regulate their buoyancy and float to the surface to form a thin "oily" looking film, or a blue-green scum several inches thick. The film may be mistaken for a paint spill. Cyanobacteria cannot maintain this abnormally high population for long and will rapidly die and disappear after 1-2 weeks. If conditions remain favorable, another bloom can rapidly replace the previous one. In fact, successive blooms may overlap so that it may appear as if one continuous bloom occurs for up to several months.

Three genera of cyanobacteria account for the vast majority of blooms, including toxic blooms, world-wide: *Microcystis*, *Anabaena* and *Aphanizomenon*. A bloom can consist of one or a mix of two or more genera of cyanobacteria.

WHERE CAN BLOOMS BE FOUND?

Blooms can occur in any nutrient-rich standing water such as lakes, ponds, roadside ditches, sewage lagoons, or overflows and embayments of rivers. Chemical and physical factors needed for a bloom to form or produce toxin are complex and uncertain. However, certain requirements are known which include elevated nutrients such as nitrogen and phosphorous (especially total phosphorous greater than 10 μ/1), pH 6-9 and temperatures which can support the relatively slow growing cyanobacteria.

Because cyanobacteria are mostly photosynthetic, they may be limited by availability of light, although they can grow under relatively low light conditions. Research has shown that some can be both photosynthetic and heterotrophic at the same time which may account for their low light tolerance.

Cyanobacteria blooms have been documented throughout Washington State. The three genera known to be the major producers of cyanobacteria blooms, including toxic blooms have been identified: *Microcystis aeruginosa*, *Anabaena flos-aquae* and *Aphanizomenon flos-aquae.*

WHEN CAN BLOOMS OCCUR?

According to scientific literature, cyanobacteria most commonly occur in late summer and early fall when water temperatures reach 72°-80° F (21°-27° C). At these temperatures cyanobacteria grow rapidly and may create a bloom within a few days. Most blooms east of the Cascade mountains in Washington probably follow the classic scenario and are visually observed in late summer and early fall.

Recent data collected in Western Washington lakes have shown cyanobacteria blooms during atypical water temperatures and times of the year. Although an individual lake in Western Washington may have a predictable bloom season, this season may vary dramatically between lakes. Based upon preliminary evidence, it is quite possible that a bloom can be found somewhere in Western Washington nearly any month of the year and at water temperatures as low as 45°-50° F (7°-10° C).

IS A PARTICULAR BLOOM TOXIC?

Not all cyanobacteria blooms are toxic. Even blooms caused by known toxin producers may not produce toxins or may produce toxins at undetectable levels. Since cyanobacteria toxins can be lethal in relatively small amounts, caution should always be taken when a bloom occurs (Table 1).

Scientists do not know what triggers toxin production by cyanobacteria. Just a few years ago, it was believed that only about 10% of all blooms produced toxins. Recent studies in Europe, Canada and United States (Wisconsin) have shown that the probability that an individual bloom containing *Anabaena, Microcystis* and/or *Aphanizomenon will* be toxic is greater than previously thought (45-75%).

Overt signs that a cyanobacteria bloom is toxic may include large numbers of dead fish, water foul or other animals within or around a body of water. Terrestrial animals found dead may have algae around the mouth area or on the feet and legs, indicating possible ingestion of and contact with a toxic bloom.

Symptoms from sublethal poisonings differ with the kind of animal, nature of toxin and quantity of toxin consumed (Table 2). Any sudden, unexplained animal illness or death occurring near a water body containing a bloom should be suspect. There have been no confirmed deaths in humans due to consumption of bloom toxins. However, some blooms have caused "outbreaks" of dermatitis (a form of "swimmers itch") and attacks of gastroenteritis in groups of swimmers. These causative agents appear not to be the same toxins as the potentially lethal toxins.

EXPERTS RECOMMEND THAT ALL CYANOBACTERIA BLOOMS BE CONSIDERED POTENTIALLY TOXIC UNTIL TESTED.

HOW DO YOU CONFIRM A TOXIC BLOOM?

Only laboratory tests can confirm whether a bloom is toxic or non-toxic. Concentrated bloom material can be collected in a clean glass or plastic bottle with a water tight lid for shipment to a laboratory equipped to test the sample. NO PRESERVATIVES SHOULD BE ADDED TO THE SAMPLE. Samples should be kept refrigerated (not frozen) and shipped with an ice pack to a testing laboratory.

Dr. Mike Crayton has been investigating toxic blooms in Washington State and has facilities for testing blooms. This test facility is currently supported by grants from the Department of Health (Office of Toxic Substances) and Department of Ecology (WQFAP). Samples should be shipped to the following address with test results expected within 24 hours after delivery. If you feel that a potential emergency may exist, samples can be picked up for same day testing. Please feel free to call the number below if you have any questions.

Ship to: Dr. M. A. Crayton
Biology Department
Pacific Lutheran University
Tacoma, Washington 98447
Tel. (253) 535-7547
FAX (253) 536-5055

ANABAENA sp.

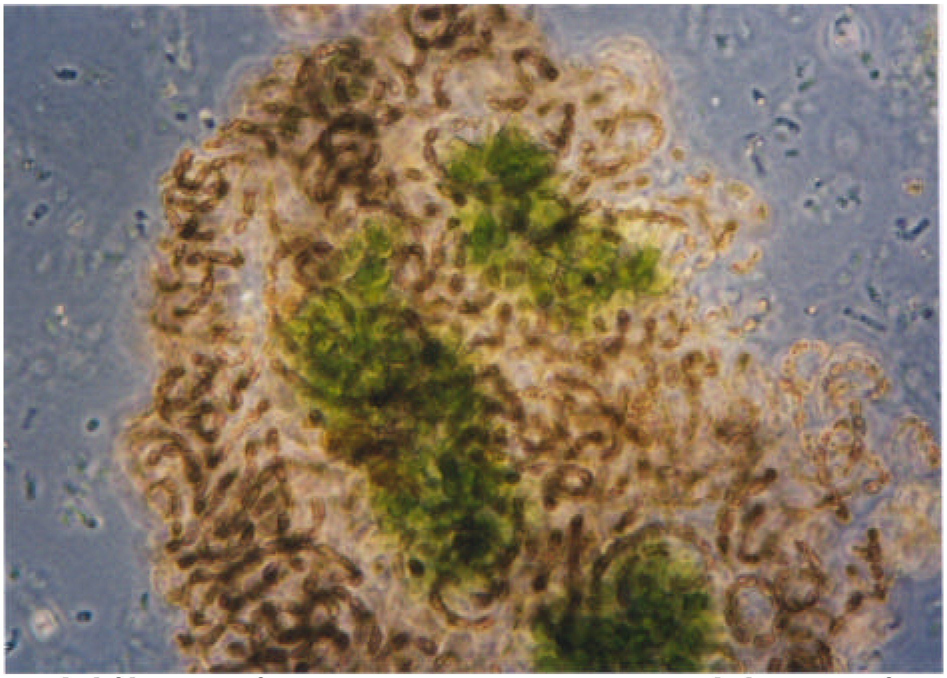

Figure 1. Entangled filaments of *Anabaena flos-aquae*. Note central clustering of green akinetes. (Phase-contrast, 160X)

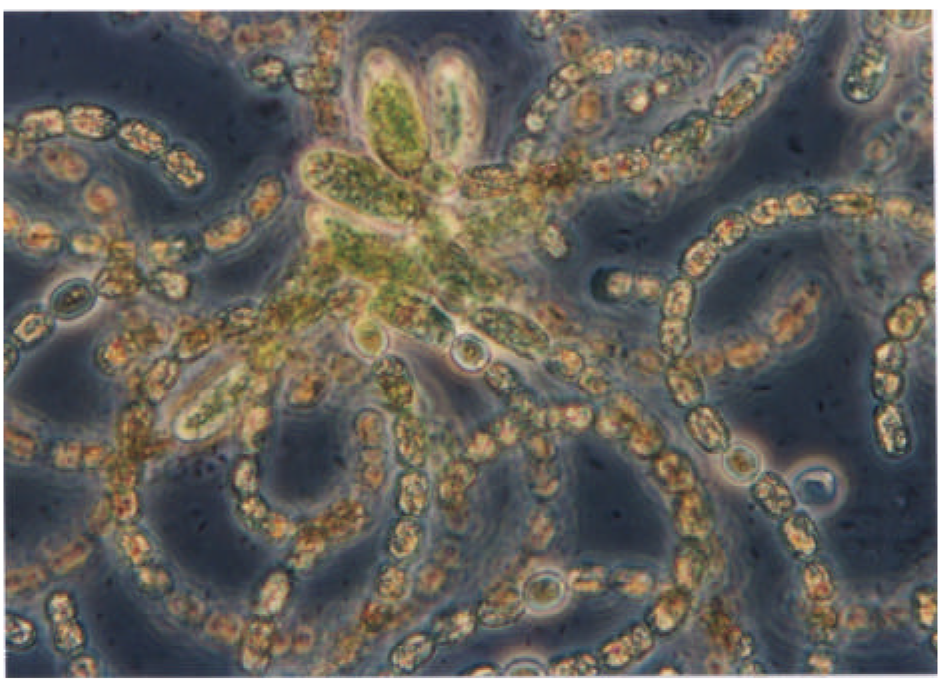

Figure 2. Filaments of *Anabaena flos-aquae* showing close-up of akinetes (upper center) and heterocysts (lower right). (Phase-contrast, 400X)

ANABAENA sp.

Description:

Cells are attached to form unbranched filaments (like a string of beads) that may appear randomly twisted and coiled, sometimes regularly coiled like springs, or entangled with numerous other filaments (Fig 1). Vegetative cells may be spherical to oblong (4-14 µm dia., 6-12 µm long) with granular contents and conspicuous, refractive pseudovacuoles. Two types of specialized cells may be present in various numbers within a filament of vegetative cells (Fig. 2). Akinetes are larger than vegetative cells and may appear spherical to sausage-shaped (6-13 µm dia., 20-50 µm long). Heterocysts, which appear "empty", are somewhat spherical (7-9 µm dia., 6-10 µm long).

Toxins:

Anabaena sp. can produce several kinds of toxins. Two different neurotoxins have been described. **Anatoxin-a** is a potent postsynaptic cholinergic nicotinic agonist, which causes a depolarizing neuromuscular blockade. **Anatoxin-a(s),** chemically unrelated to the first, acts as an inhibitor of cholinesterase leading to a neuromuscular blockade. Both cause a "tetanus-like" muscle paralysis.

Microcystins (hepatotoxins) have also been shown to be produced by members of this genus. Named for the genus in which they were originally discovered, they alter the cytoskeletal components of hepatocytes leading to intercellular dissociation causing blood accumulation within the liver and death by hypovolumic shock. Very recent experimental evidence shows that at least one of the molecular mechanisms of action is consistent with certain known carcinogens. This information has led researchers to suspect these toxins as possible liver carcinogens which could prove significant to humans following continuous, low level exposure.

Poisoning Symptoms in Animals:

Neurotoxins are notoriously rapid-acting poisons. Onset of symptoms and death to the animal may occur within a few minutes to a few hours, depending upon size of animal and amount of toxic bloom consumed. **Anatoxin-a** toxicosis may exhibit **staggering, paralysis, fasciculations** (muscle twitching), **gasping, convulsions, backward arching of neck in birds and death. Anatoxin-a(s)** induced toxicosis in experimental animals may exhibit **hypersalivation, tremors, fasciculations, involuntary muscle movement, diarrhea, cyanosis** (tongue and mouth lining appear bluish) and **death.**

Poisoning from microcystins may take 30 minutes to 24 hours to appear, depending upon the size of the animal affected and the amount of toxic bloom consumed. **Microcystin** toxicosis may exhibit **jaundice, shock, abdominal pain/distention, weakness, nausea/vomiting, severe thirst, rapid/weak pulse** and **death.**

APHANIZOMENON FLOS-AQUAE

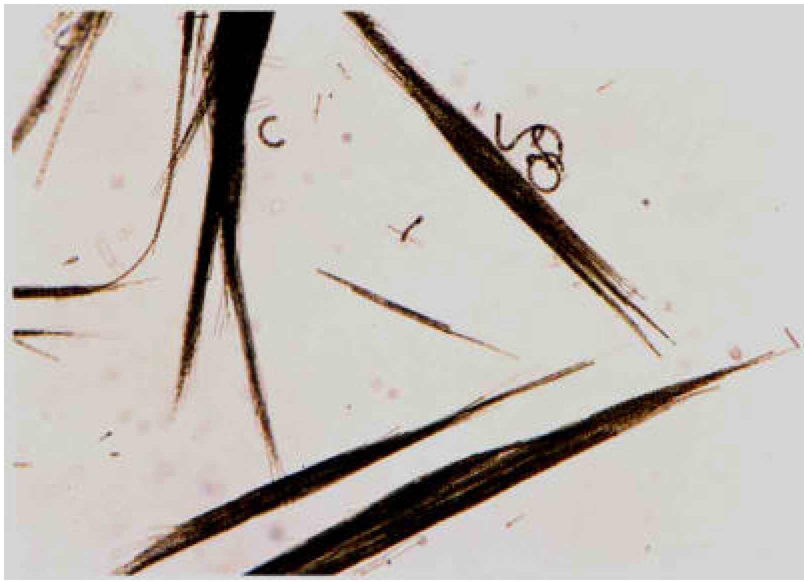

Figure 1. Characteristic clustering of *Aphanizomenon flos-aquae* filaments into parallel bundles. (Bright-field, 63X)

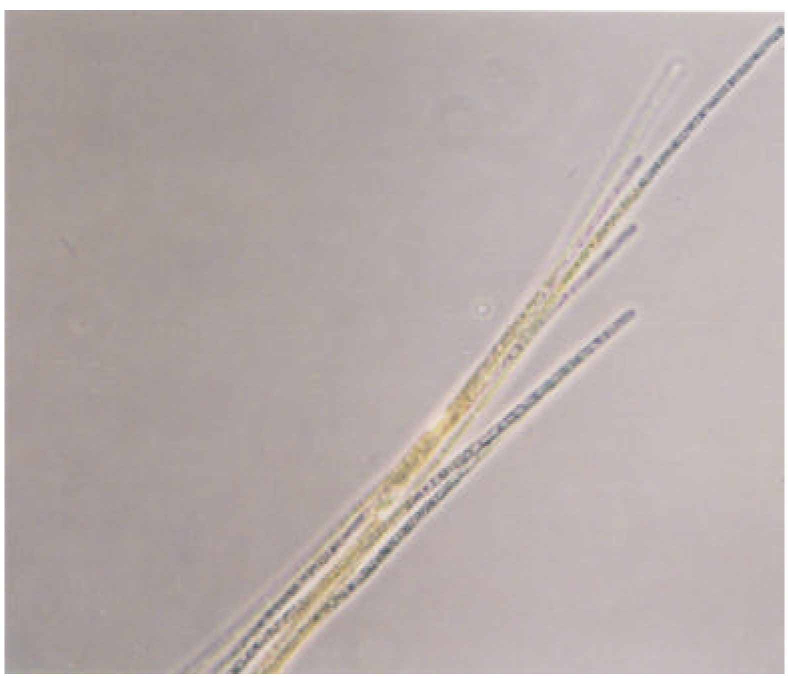

Figure 2. Individual filaments of *Aphanizomenon flos-aquae.* Centrally located akinete can be distinguished in some filaments. (Phase-contrast, 160X)

APHANIZOMENON FLOS-AQUAE

Description:

Cells united to form a straight, unbranched filament which tapers slightly toward both ends. Filaments usually clustered to form a bundle of parallel filaments (looking like a bundle of straw), which is free-floating (Fig. 1). These bundles appear to the unaided eye as prominent, blue-green, "lens-shaped bodies" suspended in the water sample. Individual cells are at least twice as long as wide (5-6 μm dia., 8-12 μm long). Each filament shows a slight tapering toward the ends with the cells near the ends being much more elongated and "empty" looking (Fig. 2). There may be one centrally located akinete and heterocyst per filament. Akinete is sausage-shaped (8 μm dia., 60-75 μm long) and located near the center of a filament. Heterocyst is oblong to cylindrical (7 μm dia, 12-20 μm long) and located in the mid-region but not adjacent to akinete. Akinete and/or heterocyst may be absent at times.

Toxins:

This organism is known to produce two of the same toxins as "Paralytic Shellfish Poison" produced by red tide organisms in marine habitats. **Saxitoxin** and **neosaxitoxin** are neurotoxins which block sodium channels of nerve cells making them incapable of generating a nerve impulse. They are effective in extremely small amounts; thus only a small amount of toxic bloom needs to be ingested to cause illness or death in animals.

Poisoning Symptoms in Animals:

Neurotoxins are notoriously rapid-acting poisons. Onset of symptoms and death to the animal may occur within a few minutes to few hours, depending upon size of animal and amount of toxic bloom consumed. **Saxitoxin/neosaxitoxin toxicosis** may exhibit **weakness, staggering, loss of muscle coordination, difficulty in swallowing, labored respiration, complete muscle paralysis** and **death**. Humans may also exhibit **tingling around the mouth and fingertips,** as well as **slurred speech**.

GLEOTRICHIA ECHINULATA

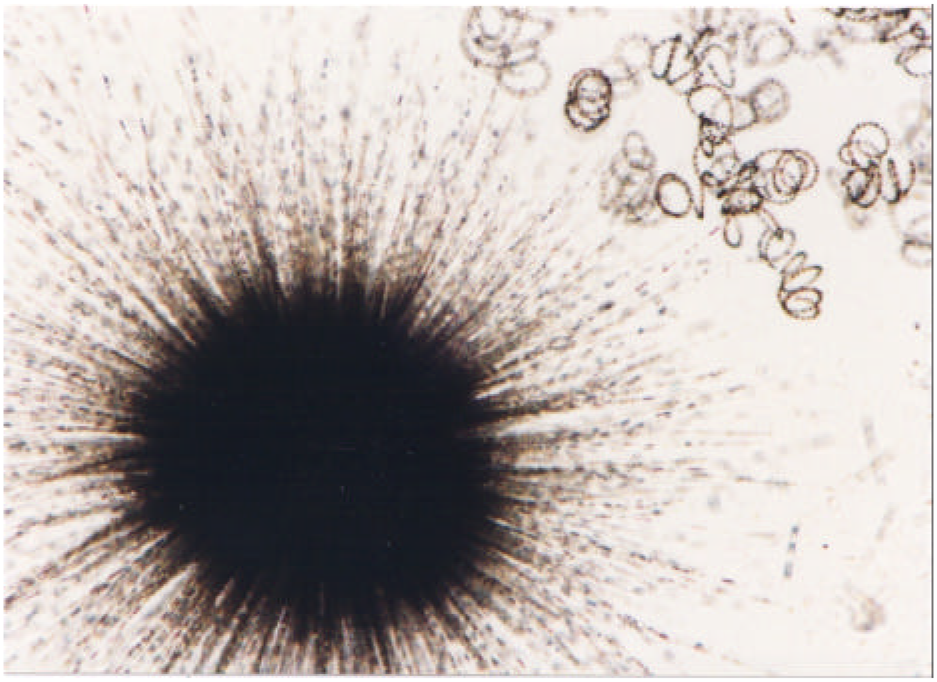

Figure 1. Colonial arrangement of filaments in *Gleotrichia echinulata*. Individual filaments radiate from a central point. (Bright-field, 63X)

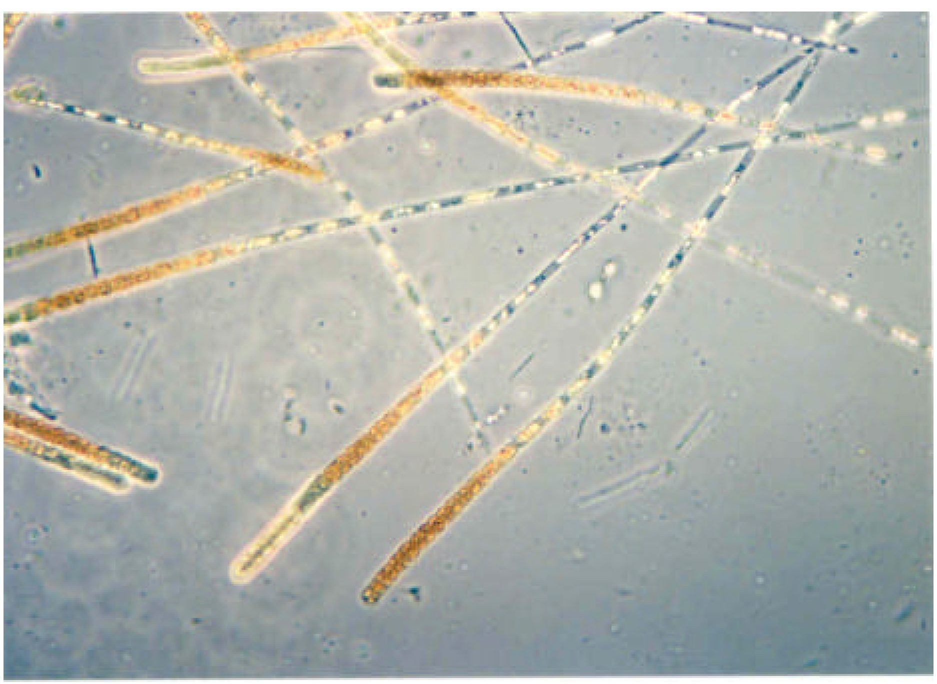

Figure 2. Individual filaments of *Gleotrichia echinulata* after gently squashing under coverslip. Although basal heterocyst is missing, a basal, sausage-shaped akinete and cells of tapering filament can be seen. (Phase-contrast, 160X)

GLEOTRICHIA ECHINULATA

Description:

Free-floating globose colony formed by numerous filaments radiating from a common center (Fig. 1). Colonies can easily be seen with the unaided eye as floating "fuzz-balls" about 2 mm (or 1/16") in diameter. Each filament tapers from a basal heterocyst into a fine hair-like tip extending beyond the mucilage which holds the colony together (Fig. 2). Vegetative cells near the base of each filament are spherical to barrel-shaped (8-10 µm dia.), becoming long and noticeably tapered at the opposite end. Highly refractive pseudovacuoles are present which allow colonies to be buoyant. The single, basal heterocyst is spherical (10 µm dia.). Akinete is adjacent to heterocyst and cylindrical (10-18 µm dia.; up to 50 µm long).

Toxin:

At least one chemically undefined **hepatotoxin** is under investigation. In addition, cell wall lipopolysaccharides have been implicated as cytotoxins, a group of toxins that are not highly lethal to animals but could account for skin irritations and outbreaks of gastroenteritis in swimmers.

Poisoning Symptoms in Animals:

Hepatotoxin toxicosis may exhibit **jaundice**, **abdominal pain/distention**, **weakness**, and **nausea/vomiting**. No animal deaths have been directly associated with this organism. **Cytotoxin** toxicosis may appear as **skin irritation** or **gastrointestinal upset** by humans swimming in water experiencing a bloom.

MICROCYSTIS AERUGINOSA

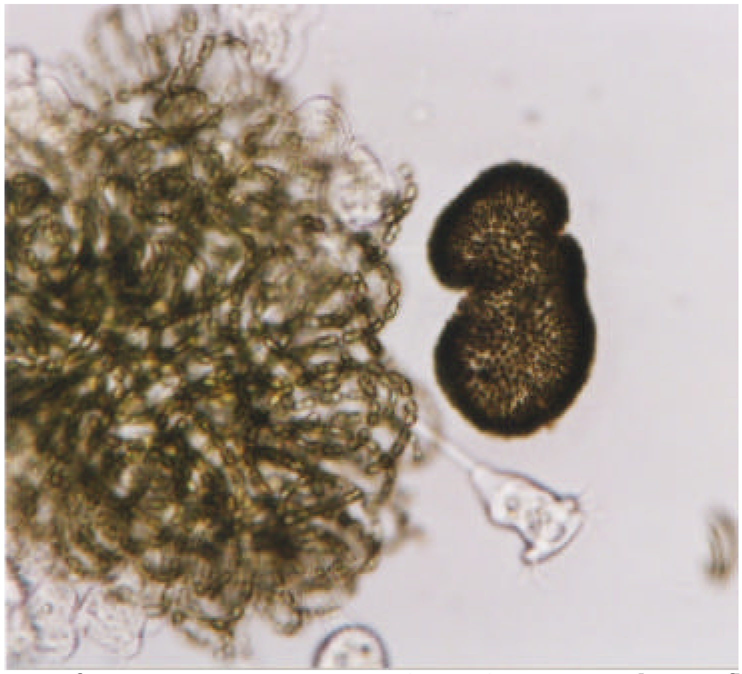

Figure 1. Lobed colony of *Microcystis aeruginosa* (center). Note *Anabaena flosaquae* (left) for size comparison of cells. (Bright-field, 160X)

Figure 2. Extensive, reticulated form of *Microcystis aeruginosa.* (Bright-field, 63X)

MICROCYSTIS AERUGINOSA

Description:

Forms clusters of cells (colonies) which may be spherical, lobed (Fig. 1) or an extensive reticulate mass (Fig. 2). Suspended colonies often appear as small blue-green "clots" to the unaided eye. Individual cells are very small (3-5 µm dia.) with conspicuous, highly refractive pseudovacuoles that cause the colonies to be buoyant and float to surface. Cells of a colony are held together by a transparent, gelatinous matrix which may be difficult to discern under microscopic examination. Akinetes and heterocysts are absent.

Toxin:

This organism is known to produce a family of toxins called **microcystins**. Named after this genus, they are heptapeptides that primarily affect the liver in animals (hepatotoxins). Current evidence suggests that the toxins alter the cytoskeletal components of hepatocytes leading to intercellular dissociation of hepatocytes causing blood accumulation within the liver and death by hypovolumic shock. Very recent experimental evidence shows that at least one of the molecular mechanisms of action is consistent with certain known carcinogens. This information has led researchers to suspect these toxins as liver carcinogens, which could prove significant to humans following continuous, low level exposure.

Poisoning Symptoms in Animals:

Symptoms may take 30 minutes to 24 hours to appear, depending upon the size of the animal affected and the amount of toxic bloom consumed. **Microcystin** toxicosis may include **jaundice**, **shock**, **abdominal pain/distention**, **weakness**, **nausea/vomiting**, **severe thirst**, **rapid/weak pulse** and **death**.

OSCILLATORIA sp.

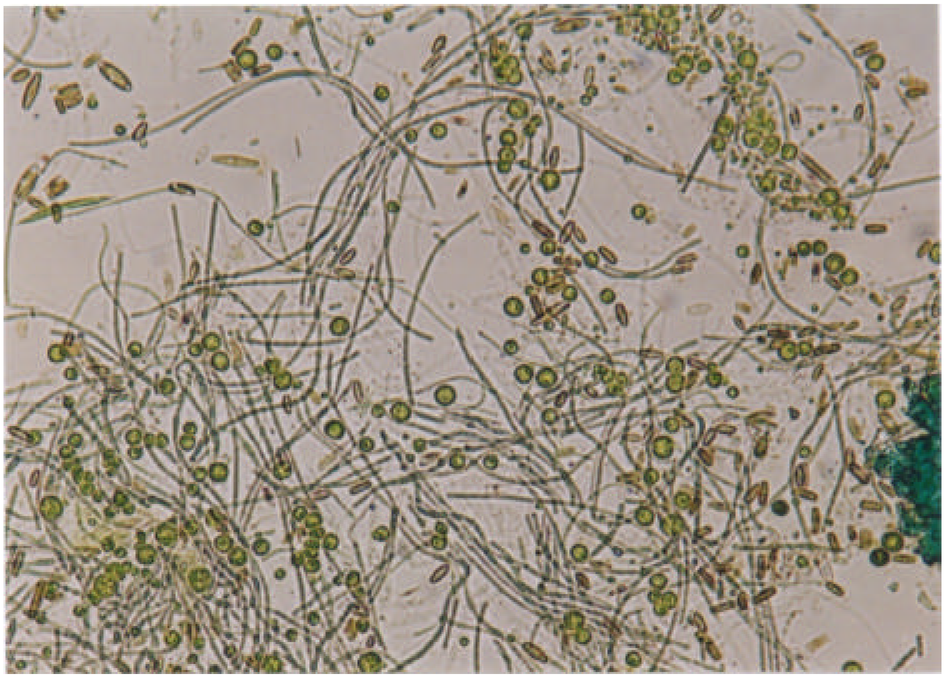

Figure 1. Thread-like filaments of a small species of *Oscillatoria* entangled with various true algae. (Bright-field, 160X)

Figure 2. Single filament of *Oscillatoria*. (Phase-contrast, 400X)

OSCILLATORIA sp.

Description:

Cells are attached to form long, straight, unbranched filaments that may be entangled to form a mass on substrates or are occasionally found free-floating. Filaments are straight throughout their length except for a possible slight tapering in the last few cells near the apex. Cell size is highly variable between species. Cells may be highly compressed against each other giving the impression of "stacked coins" or exhibit a small indentation at cell-cell juncture (Fig. 2). No akinetes nor heterocysts exist in this genus. Filaments commonly exhibit oscillating, sporadic flexing or gliding movements under the microscope, especially near the anterior ends.

Toxin:

This organism is known to produce a family of toxins called **microcystins**. They are heptapeptides that primarily affect the liver in animals (hepatotoxins) by causing liver cells to dissociate leading to blood accumulation within the liver causing death by hypovolumic shock. Recent experimental evidence shows that at least one of the molecular mechanisms of action is consistent with certain known carcinogens. This information has led researchers to suspect these toxins as liver carcinogens, which could prove significant to humans following continuous, low level exposure.

Poisoning Symptoms in Animals:

Symptoms may take 30 minutes to 24 hours to appear, depending upon the size of the animal affected and the amount of bloom consumed. **Microcystin** toxicosis may exhibit **jaundice**, **shock**, **abdominal pain/distention, weakness, nausea/vomiting, severe thirst, rapid/weak pulse** and **death.**

GLOSSARY

Akinete:	a spore produced from a vegetative cell; often considerably larger than the original vegetative cell with similar contents.
Carcinogen:	a cancer causing agent.
Colony:	a group of cells, joined together or enclosed by a common sheath or surrounding material.
Cyanosis:	a dusky bluish or purplish discoloration of skin or mucus membranes due to deficient oxygenation of the blood.
Fasciculation:	muscle twitching that involve adjoining groups of muscle fibers.
Filament:	thread-like arrangement of cells.
Globose:	globular; shaped like a sphere.
Hepatocyte:	liver cells.
Hepatotoxin:	a substance that adversely affects the liver.
Heptapeptide:	a molecule consisting of seven amino acids covalently bonded together.
Heterocyst:	a specialized, nitrogen-fixing cell that develops from a vegetative cell; usually somewhat larger than a vegetative cell with transparent contents (often appearing "empty").
Heterotrophic:	obtaining food in soluble or particulate form; not photosynthetic.
Hypovolumic shock	shock caused by decrease in volume of circulating blood.
Jaundice:	yellowish pigmentation of skin, tissues and certain body fluids due to released bile pigments; often caused by liver damage.
Neurotoxin:	any substance that is toxic to nerve cells or nerve tissue.
Oblong:	An elongated circle.
Photosynthetic:	Capable of producing food by photosynthesis.
Pseudovacuole:	False vacuoles; pockets of gas or mucilage in the cytoplasm resembling vacuoles; usually light-refracting; contribute buoyancy to cells containing them.
Reticulate:	netted; arranged in an interconnected net-work.

Spherical:	shaped like a sphere or ball.
Toxicosis:	a pathological condition caused by the action of a poison or toxin.
Vegetative cell:	typical growing and dividing cells that are not specialized for reproduction, nitrogen-fixation, etc.

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Table 1.

Toxicities of Cyanobacteria Toxins Relative to Other Biotoxins

Toxin	*Organism*	*Common Name*	*Lethal Dose (LD50)*
BOTULINUM TOXIN-a	*Clostridium botulinum*	(BACTERIUM)	0.00003
TETANUS TOXIN	*Clostridium tetani*	(BACTERIUM)	0.0001
RICIN	*Ricinus communis*	(CASTOR BEAN PLANT)	0.02
DIPHTHERIA TOXIN	*Corynebacterium diphtheriae*	(BACTERIUM)	0.3
KOKI TOXIN	*Phyllobates bicolor*	(POISON ARROW FROG)	2.7
TETRODOTOXIN	*Sphaeroides rubripes*	(PUFFER FISH)	8
SAXITOXIN	*Aphanizomenon flos-aquae*	(CYANOBACTERIUM)	9
COBRA TOXIN	*Naja naja*	(COBRA)	20
NODULARIN	*Nodularia spumigena*	(CYANOBACTERIUM)	30-50
MICROCYSTIN-LR	*Microcystis aeruginosa*	(CYANOBACTERIUM)	50
ANATOXIN-a	*Anabaena flos-aquae*	(CYANOBACTERIUM)	200
MICROCYSTIN-RR	*Microcystis aeruginosa*	(CYANOBACTERIUM)	300-600
CURARE	*Chrondodendron tomentosum*	(BRAZILIAN POISON ARROW PLANT)	500
STRYCHNINE	*Strychnos nux-vomica*	(PLANT)	500
AMATOXIN	*Amanita phalloides*	(FUNGUS)	600
MUSCARIN	*Amanita muscaria*	(FUNGUS)	1100
PHALLATOXIN	*Amanita phalloides*	(FUNGUS)	1800
GLENODIN TOXIN	*Peridinium polonicum*	(DINOFLAGELLATE ALGA)	2500
SODIUM CYANIDE			10000

*Acute LD_{50} in µg per kg bodyweight: based on intra-periotoneal injection of mice or rats

Table 2. Summary of Toxins, Their Symptoms, and Associated Genera of Cyanobacteria

TOXINS	SYMPTOMS	GENERA*
Neurotoxins	staggering, muscle twitching, gasping, convulsions, cyanosis, backward craning of neck (birds), unconsciousness, death	Anabaena Aphanizomenon Oscillatoria Trichodesmium
Hepatotoxins	weakness, anorexia, pale coloration of mucous membranes, vomiting, cold extremities, diarrhea, death	Anabaena Aphanizomenon Cylindrospermopsis Coelosphaerium Gleotrichia Microcystis Nodularia Nostoc Oscillatoria
Cytotoxins	contact skin irritation/dermatitis, gastroenteritis, diarrhea, cramps, nausea, allergic reactions	Anabaena Aphanizomenon Gleotrichia Hapalosiphon Oscillatoria Scytonema Tolypothrix

* Partial list of documented producers of toxins. Total number of known cyanobacteria that produce biotoxins is rapidly increasing as researchers continue to work in this area